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Anthocyanin and Nutritional Contents of Fermented Lebui Bean (*Cajanus* sp.) through SSF Method and Induced by *Rhizopus* sp. and *Saccharomyces* sp.

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Abstract. Anthocyanins are bioactive compounds that were very important to support health and prevent oxidation. The existence of anthocyanin in plants, one of which is characterized by the presence of their striking colors in the plant. Lebui bean (*Cajanus* sp.) have a deep black color and identified to contain anthocyanins and phenolic compounds. However, these two important bioactive compounds are not obtained in free conditions, because they were still bound in cells and cell walls through the glycoside bond, also bound to other chemical bonds in the cell. Therefore, a method needed to break the bioactive compounds from their bonds. The easiest, cheapest, and most applicable method is fermentation. Fermentation is a method of processing food that utilizes microbial functions to degrade the compounds contained in the material to produce simpler components, while at the same time breaking various binding chains between components in the material, including solid state fermentation (SSF) method. Through fermentation, it is expected that bioactive compounds including anthocyanins will be cut off and released from the bonds of glycosides and form free compounds that have functional properties. Therefore, this study aims to obtained the type of microbes (*Rhizopus* sp. and *Saccharomyces* sp.) with the most optimal fermentation time to produce fermented bean powder which has the best anthocyanin content and nutritional component. This research was statistically analysis by a nested design within two factor, the type of microbes (*Rhizopus* sp. and *Saccharomyces* sp.) as the main factor and the fermentation time (1, 2, and 3 day) was nested on the main factor. Fermentation fulfilled in two days by *Rhizopus* sp. shown the best treatment. Fermentation treatment by *Rhizopus* sp. for 1 to 3 days, produced an average level of anthocyanin in dry bases conditions is 129,81-153,55 ppm whether by *Saccharomyces* sp. was in the range of 107,12-128,11 ppm. Fermented lebui bean powder induced by *Rhizopus* sp. for 2 days fermentation has highest protein content and lowest fat content. The nutritional content of this best treatment is protein, fat, water, ash, and carbohydrate content i.e 22,39%, 0,41%, 6,73%, 3,18%, and 67,29%, respectively.

Keywords: SSF fermentation method, anthocyanin, protein, *Rhizopus* sp., *Saccharomyces* sp.

Introduction

Anthocyanins are colored water-soluble pigments which belong to widespread class of phenolic compounds i.e. flavonoid are naturally synthesized as secondary metabolism. Its common used to be colorant food that are responsible for the blue, purple, violet, magenta and red coloration [1,2], and would be replace the synthetic ones because of the negative effect when consumed by human body [3]. Due to derive from plant tissue, anthocyanins are non-toxic since it have been include in feed of animal or in food of the human in many years without generate symptoms of the diseases [4]. In addition, anthocyanins also indicate a number of merit in biological systemic, i.e. as antioxidant, anti-carcinogen, hepato protection capacity and also could improve the memory system [5,6,7].

Leguminosae or common call Fabaceae is one of anthocyanin source which abundant in Indonesia. There are classified in flowering plants family known as the legume family, bean family, pulse family or pea family, which has a large growth area mainly in Indonesia. Lebui beans – LB (*Cajanus* sp.) is the bean family that vegetate along the year and not much used in Lombok Island. In common, lebui beans were used as local dishes or just left on the field caused by limitations reference

related processing or utilization of lebui beans. Anthocyanins attached in legume could generate mostly by complicated extraction method and expensive. However, recently research as done by Mushollaeni et al., [8] has shown that by applied SSF (Solid-state Fermentation) extraction method in lebui beans would derived anthocyanin consider from lebui beans powder that contain purple color.

SSF or solid-state fermentation has been used as a potential technique based on microbial utilization to result some product such as food, feed, fuel, pharmaceutical products and industrial chemicals [9]. The application include in bioleaching, bioremediation, biobeneficiation, biopulping, etc that offer some merits. Technically, SSF is defined as the microbial cultivation method neither in lack nor nothingness of free water surroundings [10]. However, adequate moisture were further present to promote cell outgrowth. Bacteria, fungi and yeast produce vary groups of enzymes towards fermentation process. Convenient strain was select base on requirement, depend on the characteristic of the media and surrounding conditions. In this research, *Rhyzopus* sp. and *Saccharomyces cerevisae* have been choosen to ferment the lebui beans to release the bioactive compounds in this case are anthocyanins. *Rhizopus* sp is the main microorganism plays important role in tempe fermentation process. Besides, other microorganism such as yeast, i.e. *Saccharomyces cerevisae* may also contribute in fermentation process [11]. This process is easier because are not carried out under aseptic condition although starter culture is added. Moreover, the existence of those microorganism is quite abundant and cheaper. The aims of this research are to obtain the best type of microorganism both *Rhyzopus* sp. and *Saccharomyces cerevisae* to release bioactive compound such as anthocyanin through optimal fermentation time that show higher nutritional component.

2. Materials and Methods

2.2.1 Raw materials

Cajanus beans were obtained from Lombok Island, West Nusa Tenggara Indonesia. Those were sorted to remove the dirt and mold, then dried in cabinet dryer for 20 min in 40ºC up to the water rate is 12-13%. Afterwards the beans tenderly grounded and sieved 60 mesh to gain LBP (lebui bean powder).

2.2.2 SSF procedures

LBP be heated in cabinet dryer at 70-80ºC for 15 minute, then fermented for 1 to 3 days using 2% dry culture of *Rhizopus* sp. (R) or *Saccharomyces cerevisae* (S) at 27-28ºC. After fermentation, LBP was droughty applied cabinet dryer at 40ºC for 5 h, then grounded and sieved 60 mesh and stored tightly at closed jar glasses as LBP fermented.

2.2.3 General experimental procedures

This research was statistically analysis by a nested design within two factor, the type of microbes (*Rhizopus* sp. and *Saccharomyces* sp.) as the main factor and the fermentation time (1, 2, and 3 day) was nested on the main factor. Fermentation fulfilled in two days by *Rhizopus* sp. shown the best treatment. Total anthocyanin, moisture, fat, protein, ash, and carbohydrate content be used as parameters observation. The measurement of anthocyanin by UV-Visible Specstroscopy [12]. The proximate analysis measured by AOAC guidance [12], also for total anthocyanin determinedly by Juniarka et al. [13]. Moreover, ANOVA was implement to analysis the data.

3. Result and Discussion

3.1 Total Anthocyanin

The aims of the LB fermentation is to release bioactive compounds especially anthocyanins which attached inside the glycoside cells and could be used. Fermenting process in beans proven safety than applied high temperatures which could defect the compounds, moreover it may also increase the nutritional value of its. The anthocyanin levels by *Rhizopus* sp. (R) in LB were in range 129,810-153,350 ppm, whereas by *Saccharomyces serevisae* (S) were in range 107,120-128,110 ppm lower. The research shown that extension period of fermentation caused the levels of anthocyanin be decline.. In accordance to Balik [14], biochemical, degradation, metabolism, and destruction in fermentation have aims to

synthesize simply sugar compound during the microbe growth so that it would increase the mass, passingly the non-sugar compounds either secondary metabolite such as anthocyanin tend to decrease (Figure 1).

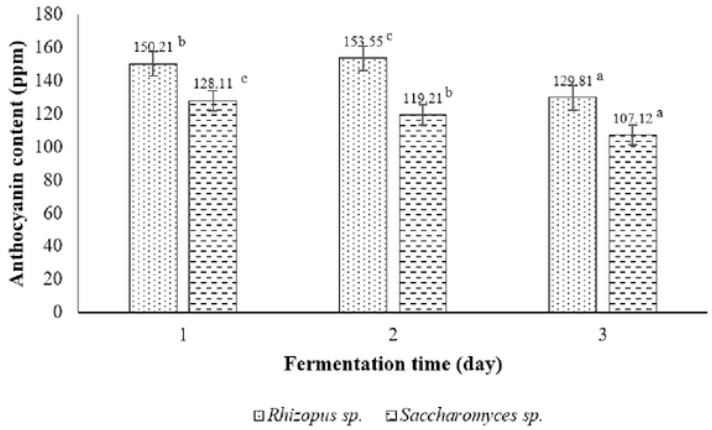

Figure 1. Comparison of nutrient content of LBP fermented by *Rhizopus* sp. and *Saccharomyces cerevisiae*

Reduction of anthocyanin throughout the fermentation also generated by Afoakwa et al. research [15]. Anthocyanin would may take lower during fermentation which is indicate by alteration of murky colour on the extract to be lighter colour. As long as an anthocyanins are belong to polyphenol components, there are easier to change by shifting of pH and temperature [16].

In fermentation process, there also include hydrolysis that causes anthocyanin shift to be anthocyanidin, then gain a simple sugar group. It's underlies the reduction in anthocyanin content during fermentation period. The decline of these anthocyanin levels continues and reached the lower peak on the 3th day of fermentation [15]. Based on this results, fermentation within in 2 days shown high level than in days 3. Alteration temperature in prolong period of fermentation would may affect degradation the level of the anthocyanin, indicated by discoloration, alteration of odor in both R and S fermentation.

3.2 Physicochemical properties

3.2.1 Protein content

Protein content of fermented LBP given averaged respectively by R is 22,39% and S is 19,09% (Table 1). Igbabul et al. [17] explained that for mahagony bean (*Afzelia africana*) fermentation more than 72 h could obtain 21.88% to 22.43-26.8% of protein. Fermentation could induce the hydrolysis of protein complex to generate simple amino acids and nitrogen compound. According to Kasprowicz-Potocka et al. [18], levels increased of this protein is influenced by biomass enhancement of microbes. Moreover, produce free amino acids and nitrogen during the fermentation were used to promote their growth. Handoyo and Morita [19], Engel et al. [20] and Meussen et al. [21] are also shown that in range pH 3,6-7, *Rhizopus* sp is optimal to growth.

3.2.2 Fat content

The fat of fermented LBP respectively in average by R is 0,41% and S is 1.25% (Table 1).. Fermentation using R or S indicate not significant distinction in fat content among 1, 2, and 3 days during fermentation. The highest average of fat content was indicate when the fermentation have been done by S within 3

days On the 3rd day, the data shown that the fat content in fermentation by S was higher is 1,30% which is signify that the number of simple fatty acid compounds have been produced by microbial hydrolysis and the breakage of the lipoprotein material, even the microbes have not been used entirely by microbes to support its metabolism [22, 23]. In other hand, fermentation by R indicate a decrease of fat content. Khetarpaul and Chauhan [24] reported that during fermentation up to 72 h, they research also signify that fat content tend to reduced. The existence of lipolytic enzymes that generated by microbes in fermentation, which is part of hydrolysis process, caused reduction levels of the fat [25].

3.3.3 Water content

This research shown that throughout the fermentation by R or S, the water content alteration in LBP related by alteration of the other nutrients value. Water content of LBP fermented respectively in average with R is 6,60-6,81% whereas fermented with S shows 9.05-9,08% higher (Figure 2). Orhevba [26] and Obadina et al. [25] explained that usually water content would decrease in the first period of fermentation, then it would increases until the end of fermentation. However, this condition is the opposite of the carbohydrate compounds that increase with reduction of water content of LBP when fermented by R with average 67,29%. This condition is conducted by Morris et al. (2004) research, which are explained that the percentage of water content was caused by relatively high of CO_2 during the fermentation process and might include in biochemical process (Table 1).

Controlling of LBP water content during fermentation was done by aeration performing in top-down reversing in every 12 h. Nout and Kiers [27] suggest to provide adequate distance between the products and regulate the thickness of the fermented product, thereby heating temperature from the *Rhizopus* sp. mass will could not reach a maximum temperature of 40-50ºC so it's still suitable for its growth. SSF method is applied to avoid the rotten of fermented LBP in high water content.

Table 1. Comparisson nutrient fermented by R and S in LBP.

Microbial	Average					
	Protein	Fat	Water	Ash	Carbohydrate	Fiber
Rhizopus sp.	22,39	0,41	6,73	3,18	67,29	32,71
Saccharomyces cerevisae	19,09	1,25	9,08	3,31	67,27	32,73

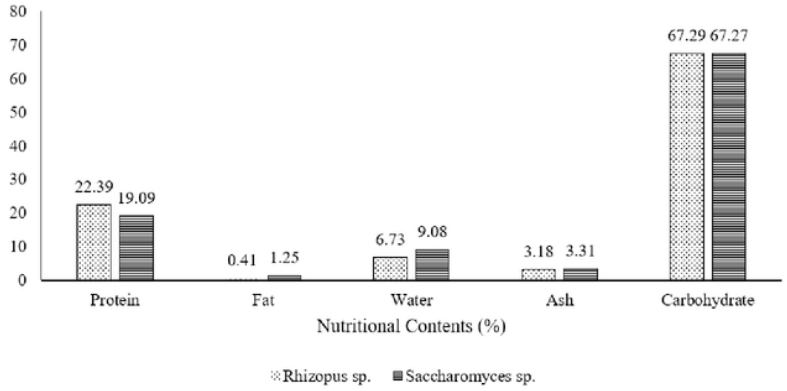

Figure 2. Comparison of nutrient content of LBP fermented by *Rhizopus* sp. and *Saccharomyces cerevisiae*

3.3.4 Ash content

Ash content (%) of fermented LBP by R are R3 <R1 <R2 is 3.21 <3.25 <3.08 <3.20 and by S are S3 <S1 <S2 is 3.34 <3.34 <3.24 (Table 1). Mostly LBP fermented by S (3.31%) shown higher content than by R (3.21%), but it is still lower than 3.5% and relatively safe based on standards for food preparations produce from the plant seeds [28]. This research was also related in other of Leguminosae group that the ash content still in ranges from 2.02-9,36% [29] and 3.53.9% [30]. Osman [31] stated that ash content in product is not positively change by fermentation. Conducted to Difo et al. [32], ash content such as Fe, Na, Mg, Zn, and K were decreased related to microbial growth.

3.3.5 Carbohydrate content

Carbohydrate in fermented LBP with R shown higher than by S, which is the average carbohydrate content respectively 67,29 and 67,27 % (Table 1). Carbohydrates levels was increased when R applied as microbial fermenter in LBP with value 66,83 to 67, 89. Whereas it will reduce from 67,33 to 67, 23 when S applied as fermenter during fermentation. Degradation trend of carbohydrate levels is also occurred in Osman [31] research. Due to enzymatic activity by microbial fermenter may cause the reduction of carbohydrate levels during fermentation. Its influence by a low pH condition which is could hinder the amylase release by microbes. Moreover, antimicrobial compounds including fermentation by microbe such as terpenoid groups was hampered.

3.3.6 Total fiber content

Total fibre content in fermented LBP shown the accretion with extention of fermentation within the end of days 3, either by R or S (Table 1). The height of total fiber in the last fermentation gave the point that cellulase from both microbes were able to digest the cell walls to be simpler coumpounds include fiber components. Related with Mirnawati et al. [33] research, which is reveals that the cellulase enzymes in plant cell wall could increase the fiber content such in palm kernel. Mirnawati et al. [34] add that by applying fungus i.e. *Eupenicillium javanicum* as fermenter, with longer fermentation would might cause the total fiber content reduction.

4. Conclusion

SSF method have mostly effect to decrease the LBP nutrient, especially anthocyanin. Even there were found increase in first day fermentation, in longer fermentation were tend to degenerate the

concentration. In fact, the reduction of anthocyanin was not quite significant from the initial nutrient of Lebui Beans. Fermentation within in days 2 by *Rhizopus* sp. shown the best treatment in this research.

5. Acknowlegement

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